FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: BMID9818US

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/807079

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/07394	(05.10.99) 5 October 1999	(08.10.98) 8 October 1998

TITLE OF INVENTION

METHOD FOR DETERMINING ALKALINE PHOSPHATASE AND ELIMINATING HAEMOGLOBIN DISTURBANCES

APPLICANT(S) FOR DO/EO/US

WEISHEIT, Ralph; and TREIBER, Wolfgang

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment. (to follow)
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

General Appointment of Representative for U.S. Patent and Trademark Office Matters; and Return postcard.

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR) 09/807079	INTERNATIONAL APPLICATION NO PCT/EP99/07394	ATTORNEY'S DOCKET NUMBER BMID9818US

21. The following fees are submitted:		CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :			
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$970.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	**$840.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$690.00**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$670.00**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	**$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =	0	x $18.00	**$0.00**	
Independent claims	- 3 =	0	x $78.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
	TOTAL OF ABOVE CALCULATIONS =			**$860.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	**$0.00**	
	SUBTOTAL =			**$860.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
	TOTAL NATIONAL FEE =			**$860.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
	TOTAL FEES ENCLOSED =			**$860.00**	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **50-0877** in the amount of **$860.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **50-0877.** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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30,444
REGISTRATION NUMBER

6 April 2001
DATE

TRANSMITTAL LETTER TO THE UNITED STATES RECEIVING OFFICE

Date	6 April 2001
International Application No.	09/807079
Attorney Docket No.	BMID9818US

I. Certification under 37 CFR 1.10 (if applicable)

JC13 Rec'd PCT/PTO 06 APR 2001

EL 841983380 US	6 April 2001
Express Mail mailing number	Date of Deposit

I hereby certify that the application/correspondence attached hereto is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.

Rose Edwards	Rose Edwards
Signature of person mailing correspondence	Typed or printed name of person mailing correspondence

II. ☐ New International Application

TITLE		Earliest priority date (Day/Month/Year)

SCREENING DISCLOSURE INFORMATION: In order to assist in screening the accompanying international application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied. (Note: check as many boxes as apply):

A. ☐ The invention disclosed was **not** made in the United States.

B. ☐ There is no prior U.S. application relating to this invention.

C. ☐ The following prior U.S. application(s) contain subject matter which is related to the invention disclosed in the attached international application. *(NOTE: priority to these applications may or may not be claimed on form PCT/RO/101 (Request) and this listing does not constitute a claim for priority).*

application no.		filed on	
application no.		filed on	

D. ☐ The present international application ☐ is identical ☐ contains less subject matter than that found in the prior U.S. application(s) identified in paragraph C.

E. ☐ The present international application ☐ contains additional subject matter not found in the prior U.S. application(s) identified in paragraph C. above. The additional subject matter is found on pages ____ and ☐ **DOES NOT ALTER** ☐ **MIGHT BE CONSIDERED TO ALTER** the general nature of the invention in a manner which would require the U.S. application to have been made available for inspection by the appropriate defense agencies under 35 U.S.C. 181 and 37 CFR 5.1. See 37 CFR 5.15

III. ☐ A Response to an Invitation from the RO/US. The following document(s) is (are) enclosed:

A. ☐ A Request for An Extension of Time to File a Response

B. ☐ A Power of Attorney (General or Regular)

C. ☐ Replacement pages:

pages		of the request (PCT/RO/101)	pages		of the figures
pages		of the description	pages		of the abstract
pages		of the claims			

D. ☐ Submission of Priority Documents

Priority document		Priority document	

E. ☐ Fees as specified on attached Fee Calculation sheet form PCT/RO/101 annex

IV. ☐ A Request for Rectification under PCT 91 ☐ **A Petition** ☐ **A Sequence Listing Diskette**

V. ☒ Other (please specify): Transmittal Letter to the United States Designated/Elected Office (DO/EO/US) Concerning a Filing Under 35 U.S.C. 371

The person signing this form is the:		
☐	Applicant	Marilyn L. AMICK
☒	Attorney/Agent (Reg. No.) 30,444	Typed name of signer
☐	Common Representative	Marilyn Amick
		Signature

09/807079
JC02 Rec'd PCT/PTO 06 APR 2001

Method for determining alkaline phosphatase and eliminating haemoglobin disturbances

The invention concerns a method for the determination of alkaline phosphatase in a sample by optical measurement in which interference by free haemoglobin or blood substitutes is eliminated by means of certain wavelength combinations, a method for eliminating interference caused by free haemoglobin or blood substitutes in a determination of alkaline phosphatase and the use of certain wavelength combinations to eliminate interference by free haemoglobin or blood substitutes.

It is known that haemolysis considerably interferes with some diagnostic methods for the determination of analytes. Haemolysis is understood as any destruction of erythrocytes for example by mechanical, osmotic, chemical or enzymatic action on the cell membrane of the erythrocytes. As a result of haemolysis, the blood pigment haemoglobin (Hb) is released and can no longer be removed from a sample. The presence of haemoglobin is problematic because, on the one hand, the absorption spectrum of haemoglobin in some cases overlaps considerably with the spectra of the substances to be detected and indicators (chromogens) which can result in measuring errors in photometric tests. On the other hand, haemoglobin can also react chemically with sample components to form substances which can also result in false measurements.

Recently blood substitutes whose manufacture is based on

haemoglobin are being used more and more frequently for therapeutic purposes for example after a large loss of blood. The haemoglobin in blood substitutes can be native or synthetic. Often Hb-like compounds are also used. In contrast to haemolysis, the Hb content in blood, serum or plasma may be more than 2000 mg/dl during treatment with blood substitutes. Hence interference in samples which contain blood substitutes is often considerably more pronounced than in haemolytic samples since the haemoglobin or the synthetic analogue is in a free form right from the beginning.

Interference by free haemoglobin is particularly serious in the photometric determination of alkaline phosphatase. The formation of 4-nitrophenol is measured at 415 nm (increase of absorbance) for the determination of alkaline phosphatase. Haemoglobin also absorbs at 415 nm. The presence of haemoglobin interferes with the determination of alkaline phosphatase in two respects: On the one hand the Hb spectrum changes in a time-dependent manner (increase of absorbance) in an alkaline medium, on the other hand, the photometer limit of the measuring instrument is reached above a certain Hb content.

Various methods have been published in the prior art to eliminate the spectral and chemical influence of haemoglobin on the analysis of serum or plasma samples.

Due to the simple handling on automated analyzers, a second measuring wavelength (secondary wavelength) is often used in addition to the first measuring wavelength (main wavelength) in order to eliminate the interfering effect of interfering substances such as haemoglobin,

bilirubin and lipaemia or to at least minimise this effect. In Clin. Chem. 25/6, 951-959 (1979) Hahn et al mention that the secondary wavelength should be selected such that it is near to the absorption minimum of the chromogen and near to the absorption maximum of the interfering substance. However, it is not possible to use the stated measuring procedures to eliminate interference in the determination of alkaline phosphatase.

Jay and Provasek describe in Clin. Chem. 39/9, 1804-1810 (1993) that haemoglobin interference of the alkaline phosphatase determination is caused by a time-dependent change of the Hb spectrum. This interference can be eliminated by mathematical correction algorithms (determination of the Hb concentration in the sample and correction of the measured value for alkaline phosphatase by a certain amount that is equivalent to the measured amount of Hb).

Although the mathematical correction mentioned by Jay and Provasek eliminates the influence of Hb up to at least 800 mg/dl Hb, it is, however, not very user-friendly since it requires an additional measurement of the Hb content and subsequently an additional mathematical correction step.

Jay and Provasek (supra) describe a further method for eliminating interference by the so-called rate-blank measurement. The correction of haemolysis interference by rate-blank measurements is also described in EP-A-0 695 805. In this method the sample is subjected to a pre-reaction to determine the degree of haemolysis of the sample before the actual photometric determination

of a component contained in the sample. The measured value obtained subsequently is then corrected by a value which has been determined by correlating the degree of haemolysis with the amount by which the interfering components contribute to the measuring error.

Hb interference can be eliminated by rate-blank measurements but only up to a Hb content of ca. 1200 mg/dl since the photometer limit is reached at higher Hb contents. This may be adequate for eliminating haemolysis interference but it is not sufficient at all for eliminating interference by blood substitutes.

Another method for eliminating haemoglobin interference was published for the determination of albumin (PCT application WO 97/45728) in which an elimination of haemoglobin interference was achieved by special combinations of main and secondary wavelengths. However, the wavelength combinations mentioned in this PCT application cannot be used for the determination of alkaline phosphatase since a measuring signal would no longer be obtained for 4-nitrophenol at these wavelengths.

The laid-open publication WO 97/45733 describes that interference by haemoglobin can be eliminated by using the wavelengths 546 and 570 in individual UV tests. However, this method can only be used for enzymatic UV tests with a main measurement wavelength of 340 nm. Although a complete elimination of Hb interference can be achieved solely by the use of the secondary wavelengths 546 or 570 nm, this is not possible for enzymatic chromogenic tests such as the determination of alkaline phosphatase in which the main measurement

wavelength is in the range of 415 nm.

The US patent 5,766,872 mentions that a secondary wavelength of 577 nm reduces haemolysis interference in the amylase determination. However, the quoted measurement data show that there is already a significant deviation of the measured values of up to 8 % at a Hb content of 500 mg/dl. This may be sufficient to eliminate haemolysis interference but it is probable that at higher Hb concentrations (such as those which occur during treatment with blood substitutes) this deviation of the measured values would become larger due to the use of a main measurement wavelength of ca. 415 nm and that there would no longer be an adequate elimination of Hb interference.

No method for the determination of alkaline phosphatase is known in the prior art which can also be carried out without interference in the presence of high concentrations of Hb such as those which occur in samples containing blood substitutes.

The object was therefore to develop an improved method for the determination of alkaline phosphatase in a sample which largely overcomes the disadvantages of the prior art. In particular it is intended to provide a simple and user-friendly method for eliminating interference by haemoglobin and by blood substitutes based on haemoglobin when determining alkaline phosphatase.

The object is achieved by a method described in more detail in the claims for the determination of alkaline phosphatase in a sample by optical measurement. The

method is characterized in that 450 ± 10 nm is used as a main measurement wavelength and at least one of the wavelengths 480 ± 10 nm, 546 ± 10 nm or 575 ± 10 nm is used as the secondary measurement wavelength. Preferably only one of the said secondary measurement wavelengths is used.

It surprisingly turned out that Hb interference of the determination of alkaline phosphatase can be effectively eliminated when the main wavelength and also the secondary wavelength is changed. It is not sufficient for a satisfactory elimination of Hb interference to only change the main wavelength or only the secondary wavelength.

Due to the absorption spectrum of 4-nitrophenol it is possible to measure alkaline phosphatase not only at 415 nm but also at 450 ± 10 nm. Although the main measurement wavelength is then not in the usual absorption maximum of the detection reaction but on its flank, the measured signal obtained is nevertheless adequate for an exact determination of alkaline phosphatase.

The selection of the new main measurement wavelength of 450 ± 10 nm already leads to a slight reduction of the haemoglobin interference, but a complete elimination of interference is surprisingly only obtained by combining the main wavelength 450 ± 10 nm with at least one of the secondary wavelengths 480 ± 10 nm, 546 ± 10 nm or 575 ± 10 nm. A secondary wavelength of 570 nm has proven to be particularly suitable. The secondary wavelength of 480 ± 10 nm has proven to be very suitable for eliminating Hb interference especially for the determination of

alkaline phosphatase according to the IFCC method (example 2).

Other secondary wavelengths such as 340, 376, 505, 600, 660 and 700 nm have proven to be unsuitable for eliminating haemoglobin interference.

The method according to the invention enables interference of the alkaline phosphatase determination by haemoglobin or haemoglobin-like compounds to be eliminated for the first time in a simple manner up to a Hb content of at least 3000 mg/dl. The upper limit for the elimination of Hb interference is the limit determined by the performance of the photometer. Hence the method according to the invention can be expected to achieve a good elimination of interference up to 6500 mg/dl haemoglobin content. Furthermore the invention can be applied to the various reagents for the determination of alkaline phosphatase as shown in examples 1 to 3.

The method according to the invention is suitable for a determination of any samples in which free haemoglobin is present. The term free haemoglobin in the sense of the invention is used to distinguish it from haemoglobin which is present in intact erythrocytes. Examples of samples that contain free haemoglobin are haemolytic serum or plasma samples or samples which contain blood substitutes. Examples of blood substitutes that fall under the term free haemoglobin in the sense of the present invention are derivatized, polymerized, modified or cross-linked derivatives of haemoglobin and in particular human haemoglobin or bovine haemoglobin e.g. DCL haemoglobin (diaspirin-crosslinked haemoglobin) or recombinantly produced haemoglobin.

The invention also concerns a method for eliminating interference caused by free haemoglobin in a method for determining alkaline phosphatase. The method is characterized in that 450 ± 10 nm is used as a main measurement wavelength and at least one of the wavelengths 480 ± 10 nm, 546 ± 10 nm or 575 ± 10 nm is used as a secondary measurement wavelength.

A further subject matter of the invention is the use of a main measurement wavelength of 450 ± 10 nm in combination with at least one of the secondary measurement wavelengths 480 ± 10 nm, 546 ± 10 nm or 575 ± 10 nm to eliminate interference by free haemoglobin or by blood substitutes manufactured on the basis of haemoglobin in a method for determining alkaline phosphatase.

The invention is elucidated by the following examples:

Examples

General Methods:

A solution containing Hb was added to a part of a serum pool to yield a Hb content of at least 3000 mg/dl. Another part of the same serum pool of the same volume was admixed with an equivalent amount of NaCl solution (154 mmol/l). Both parts were subsequently mixed with one another in different ratios to obtain a Hb concentration series of 11 samples with no Hb in the lower sample and at least 3000 mg/dl Hb in the highest sample.

Example 1
Determination of alkaline phosphatase according to the SFBC method

Determination according to the recommendation of the Société Française de Biologie Clinique according to Ann. Biol. Clin. Vol. 35, 271 (1977)

The determination was carried out on a Boehringer Mannheim/Hitachi 911 analyzer.

The following reagents were used:
reagent 1: 930 mmol/l 2-amino-2-methyl-1-propanol buffer pH 10.5;
1.03 mmol/l magnesium aspartate
reagent 2: 930 mmol/l 2-amino-2-methyl-1-propanol buffer, pH 10.5;
1.03 mmol/l magnesium aspartate;
98 mmol/l 4-nitrophenyl phosphate

The test procedure was as follows: 250 µl reagent 1 was added to 11 µl sample and after 5 min 50 µl reagent 2 was added. The analyte was determined after a further 50 sec over a period of 4 min during which the change in absorbance was measured during this 4 min. Combinations of the following main measurement wavelengths (λ_1) and secondary measurement wavelengths (λ_2) were used for the measurement: λ_1/λ_2 = 415/546 nm, 415/570 nm, 415/660 nm and 450/660 nm, (comparison) and 450/546 nm and 450/570 nm (invention).

Alkaline phosphatase was determined by the rate blank measurement mentioned by Jay and Provasek as a further comparison (referred to as 415/660 nm RB).

The result is shown in table 1. It can be seen that when using the inventive measurement wavelength combinations of 450/546 nm or 450/570 nm the effect of haemoglobin is significantly reduced compared to the other measurement wavelength combinations or compared to the rate blank measurement.

Table 1

Measured content of alkaline phosphatase at 37°C in U/l

Hb content* [mg/dl]	415/546 nm	415/570 nm	415/660 nm	415/660 nm RB	450/660 nm	450/546 nm	450/570 nm
0	42	42	42	42	42	41	42
300	33	32	32	44	35	40	41
600	24	24	22	46	29	40	40
900	16	16	14	46	24	39	41
1200	11	11	8	44	22	40	40
1500	7	7	3	5	19	40	40
1800	2	2	-2	0	17	39	40
2100	3	3	-2	0	17	40	41
2400	3	3	-2	1	15	43	42
2700	4	3	-1	0	16	42	43
3000	4	3	-2	1	19	45	45

* in this case a cross-linked haemoglobin was used.

Example 2
Determination of alkaline phosphatase according to the IFCC method

Determination according to the recommendations of the International Federation of Clinical Chemistry according to J. Clin. Chem. Clin. Biochem. vol. 21, 731-748 (1983).

The determination was carried out on a Boehringer Mannheim/Hitachi 911 analyzer.

The following reagents were used:
reagent 1: 360 mmol/l 2-amino-2-methyl-1-propanol buffer pH 10.4 (30°C);
2.04 mmol/l magnesium acetate; 1.02 mmol/l zinc sulphate;
2.04 mmol/l N-(2-hydroxyethyl)-ethylenediamine triacetic acid)
reagent 2: 360 mmol/l 2-amino-2-methyl-1-propanol buffer, pH 10.4 (30°C);
2.04 mmol/l magnesium acetate; 1.02 mmol/l zinc sulphate;
2.04 mmol/l N-(2-hydroxyethyl)-ethylenediamine triacetic acid)
104 mmol/l 4-nitrophenyl phosphate

The test procedure was as follows: 250 µl reagent 1 was added to 7 µl sample and after 5 min 60 µl reagent 2 was added. The analyte was determined after a further 50 sec over a period of 4 min during which the change in absorbance was measured during this 4 min. Combinations of the following main measurement wavelengths (λ_1) and secondary measurement wavelengths (λ_2) were used for the

measurement: λ_1/λ_2 = 415/700 nm (comparison) and 450/480 nm (invention).

The result is shown in table 2. It can be seen that when using the inventive measurement wavelength combination of 450/480 nm the effect of haemoglobin is significantly reduced compared to the previous measurement wavelength combination 415/700 nm and no negative values occur even at very high Hb contents.

Table 2

Measured content of alkaline phosphatase at 37°C in U/l

Hb content* [mg/dl]	415/700 nm	450/480 nm
0	167	170
300	157	164
600	152	160
900	145	157
1200	139	154
1500	133	151
1800	126	149
2100	123	150
2400	116	153
2700	-4.3	154
3000	-4.4	153

* in this case a recombinantly produced haemoglobin was used.

Example 3
Determination of alkaline phosphatase according to the DGKC method

Determination according to the recommendations of the "Deutsche Gesellschaft für Klinische Chemie" according to Z. Klin. Chem. Klin. Biochem. vol. 10, 290 (1972).

The determination was carried out on a Boehringer Mannheim/Hitachi 911 analyzer.

The following reagents were used:
reagent 1: 1.02 mmol/l diethanolamine buffer, pH 9.8;
0.51 mmol/l magnesium chloride,
reagent 2: 1.02 mmol/l diethanolamine buffer, pH 9.8;
0.51 mmol/l magnesium chloride;
61 mmol/l 4-nitrophenyl phosphate

The test procedure was as follows: 250 µl reagent 1 was added to 4 µl sample and after 5 min 50 µl reagent 2 was added. The analyte was determined after a further 50 sec over a period of 4 min during which the change in absorbance was measured during this 4 min. Combinations of the following main measurement wavelengths (λ_1) and secondary measurement wavelengths (λ_2) were used for the measurement: λ_1/λ_2 = 415/700 nm (comparison) and 450/546 nm (invention).

The result is shown in table 3. It can be seen that when using the inventive measurement wavelength combination of 450/546 nm the effect of haemoglobin is significantly reduced compared to the previous measurement wavelength combination 415/700 nm and no negative values occur even at very high Hb contents.

Table 3

Measured content of alkaline phosphatase at 37°C in U/l

Hb content* [mg/dl]	415/700 nm	450/546 nm
0	287	298
650	226	279
1300	172	277
1950	119	278
2600	65	278
3250	20	278
3900	-25	280
4550	-65	284
5200	-100	286
5850	-13	286
6500	-15	302

* in this case a polymerized haemoglobin was used.

Claims

1. Method for the determination of alkaline phosphatase in a sample by optical measurement, wherein 450 $\pm$ 10 nm is used as a main measurement wavelength and at least one of the wavelengths 480 $\pm$ 10 nm, 546 $\pm$ 10 nm or 575 $\pm$ 10 nm is used as the secondary measurement wavelength.

2. Method as claimed in claim 1, wherein 480 $\pm$ 10 nm is used as the secondary measurement wavelength.

3. Method as claimed in claim 1, wherein 546 $\pm$ 10 nm is used as the secondary wavelength.

4. Method as claimed in claim 1, wherein 575 $\pm$ 10 nm is used as the secondary wavelength.

5. Method as claimed in claim 1, wherein 570 nm is used as the secondary wavelength.

6. Method as claimed in one of the previous claims, wherein the determination is carried out in a serum or plasma sample.

7. Method as claimed in one of the previous claims, wherein a sample is determined which contains free haemoglobin or a blood substitute manufactured on a haemoglobin basis.

8. Method as claimed in claim 7, wherein the blood substitute contains a derivatized, polymerized, modified or cross-linked human haemoglobin, bovine haemoglobin or a recombinantly produced haemoglobin.

9. Method as claimed in one of the previous claims, wherein the sample has a haemoglobin content of up to 6500 mg/dl.

10. Method for eliminating interference caused by free haemoglobin or blood substitutes in a method for determining alkaline phosphatase, wherein a main measurement wavelength of 450 ± 10 nm is used and at least one of the wavelengths 480 ± 10 nm, 546 ± 10 nm or 575 ± 10 nm is used as a secondary measurement wavelength.

11. Use of a main measurement wavelength of 450 ± 10 nm in combination with at least one of the secondary measurement wavelengths 480 ± 10 nm, 546 ± 10 nm or 575 ± 10 nm to eliminate interference by free haemoglobin or by blood substitutes in a method for determining alkaline phosphatase.

Abstract

The invention concerns a method for the determination of alkaline phosphatase in a sample by optical measurement in which interference by free haemoglobin or blood substitutes is eliminated by means of certain wavelength combinations, a method for eliminating interference caused by free haemoglobin or blood substitutes in a determination of alkaline phosphatase and the use of certain wavelength combinations to eliminate interference by free haemoglobin or blood substitutes.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Assistant Commissioner for Patents
Washington, DC 20231

GENERAL APPOINTMENT OF REPRESENTATIVE FOR U.S. PATENT AND TRADEMARK OFFICE MATTERS

The undersigned applicant or assignee hereby appoints D. Michael Young, Reg. No. 33,819, Richard T. Knauer, Reg. No. 35,575, Brent A. Harris, Reg. No. 39,215, Kenneth J. Waite, Reg. No. 45,189, and Marilyn L. Amick, Reg. No. 30,444 all of Roche Diagnostics Corporation, 9115 Hague Road, P.O. Box 50457, Indianapolis, Indiana 46250, Telephone No. (317) 845-2000, and Jill Lynn Woodburn, Reg. No. 39,874 of The Law Office of Jill L. Woodburn, L.L.C., 6633 Old Stonehouse Drive, Newburgh, Indiana 47630-1785, Telephone No. (812) 842-2660:

to prosecute and transact all business on its behalf before the United States Patent and Trademark Office in connection with any U.S. patent assigned to it and any U.S. patent application filed by it or on its behalf and to receive payments on its behalf.

Signed this 18th day of September, 2000 at Mannheim, Germany.

Roche Diagnostics GmbH

Signature

Dr. Michael Jung
Print Name

Director
Position or Title

Roche Diagnostics GmbH

Signature

Dr. Manfred Weber
Print Name

Senior Director
Position or Title

G:\CORE\IPLD\FORMS\APPOINTMENT.doc

Docket No.

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD FOR DETERMINING ALKALINE PHOSPHATASE AND ELIMINATING HAEMOGLOBIN DISTURBANCES

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on October 05, 1999 as United States Application No. or PCT International Application Number PCT/EP99/07394

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims. as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
198 46 301.4	DE	08 October 1998	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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1-00

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DECLARATION

I, Sabine Frieda Katharina Town, declare that I am a citizen of the Federal Republic of Germany, residing at Waldstraße 45, 82386 Oberhausen, Federal Republic of Germany, that I am fluent in German and English, that I am a competent translator from German into English and that the attached is a true and accurate translation made by me into the English language of International Patent Application No. PCT/EP99/07394 dated 05.10.1999.

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

I hereby subscribe my name to the foregoing declaration, this eighteenth day of January 2001.

Sabine F.K. Town